Cell Metabolism
Perspective

Lipid Sensing and Insulin Resistance in the Brain

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Lipid sensing and insulin signaling in the brain independently triggers a negative feedback system to lower glucose production and food intake. Here, we discuss the underlying molecular and neuronal mechanisms of lipid sensing and insulin signaling in the hypothalamus and how these mechanisms are affected in response to high-fat feeding. We propose that high-fat feeding concurrently disrupts hypothalamic insulin-signaling and lipid-sensing mechanisms and that experiments aimed to restore both insulin action and lipid sensing in the brain could effectively lower glucose production and food intake to restore metabolic homeostasis in type 2 diabetes and obesity.

Introduction

The nutritional status of the body is detected by the central nervous system, where it orchestrates and relays signals in various brain nuclei and triggers peripheral responses to maintain metabolic homeostasis. These peripheral responses include behavioral effects such as feeding, as well as fuel mobilization and utilization or fuel storage (Morton et al., 2006; Lam, 2010).

Hepatic glucose production and food intake are the key contributors to glucose and energy homeostasis. Dysregulation of hepatic glucose production and feeding partly leads to fasting hyperglycemia and energy imbalance as seen in type 2 diabetes and obesity. It is now recognized that the brain, primarily hypothalamic nuclei but also other loci in the midbrain and hindbrain, detects nutrients and nutritionally regulated hormonal cues such as insulin, leptin, glucagon-like peptide 1, and ghrelin to regulate energy balance and glucose homeostasis (Wiedmer et al., 2007; Barrera et al., 2011; Morton et al., 2006; Lam et al., 2009). In this perspective, we will direct our focus to lipid sensing and insulin signaling in the brain.

Insulin signaling and lipid sensing in the hypothalamus independently triggers a negative feedback system to inhibit glucose production and appetite (Caspi et al., 2007; Obici et al., 2002c; Schwartz and Porte, 2005). We here discuss the underlying molecular and neuronal mechanisms of lipid sensing and insulin signaling in the hypothalamus and how these mechanisms are affected in response to high-fat feeding. We propose that high-fat feeding concurrently disrupts hypothalamic insulin signaling and lipid sensing mechanisms and dysregulates glucose production and feeding.

Lipid Sensing in the Brain

Fatty acids are used by neurons for the synthesis of membranes and are commonly not used by the brain as a fuel. However, short-term accumulation of fatty acid-derived molecules in the brain has been proposed to serve as a signal of nutrient abundance and trigger negative feedback systems to limit the entering of endogenous and exogenous-derived nutrients into circulation. Based on studies spanning a decade, a lipid-sensing pathway has emerged in the brain that limits the endogenous output of glucose into circulation by the liver (Figure 1).

Long-Chain Fatty Acyl-CoA

Short-term intracerebroventricular (third) administration of long-chain fatty acids (LCFAs)-oleic acids into conscious, unrestrained rats inhibits the rate of glucose production in an experimental pancreatic clamp setting by which circulating insulin levels are maintained at basal level (Obici et al., 2002b). The experimental procedure of the pancreatic (basal insulin)-euglycemic clamp in combination with tracer-dilution methodology is used to evaluate whether selective activation of central nervous system (CNS) lipid sensing alter glucose kinetics. This procedure enables the evaluation of steady-state changes in the rate of glucose production and utilization in response to experimental treatments (i.e., direct delivery of reagents into the hypothalamus to selectively alter lipid sensing mechanisms). Importantly, glucoregulatory hormones such as insulin are maintained at basal levels, and thus any detected changes in glucose kinetics are due directly to the treatments rather than changes in circulating glucoregulatory hormones. CNS lipid sensing is then evaluated in the context when insulin signaling is not stimulated in the brain. Central oleic acid is equally effectively to lower plasma glucose levels in a nonclamp postprandial setting (Obici et al., 2002b), suggesting that the inhibition of glucose production induced by central oleic acid lowers plasma glucose levels. This study is the first to support a potential metabolic impact of CNS lipid sensing, but does the brain senses a physiological rise in fatty acids in blood circulation to regulate glucose homeostasis?

The mechanism by which circulating fatty acids cross the blood-brain barrier remains elusive. The blood-brain barrier is once thought to be impermeable to fatty acids (Pardridge and Oldendorf, 1977) but later studies challenged this hypothesis since systemically administered radiolabeled fatty acids incorporate into the brain (Miller et al., 1987; Freed et al., 1994). Circulating fatty acids access the brain by passive diffusion (Hamilton and Brunaldi, 2007) or by translocation via carrier proteins such as CD36 and FATP1 (Mitchell et al., 2009). Upon entry into glial

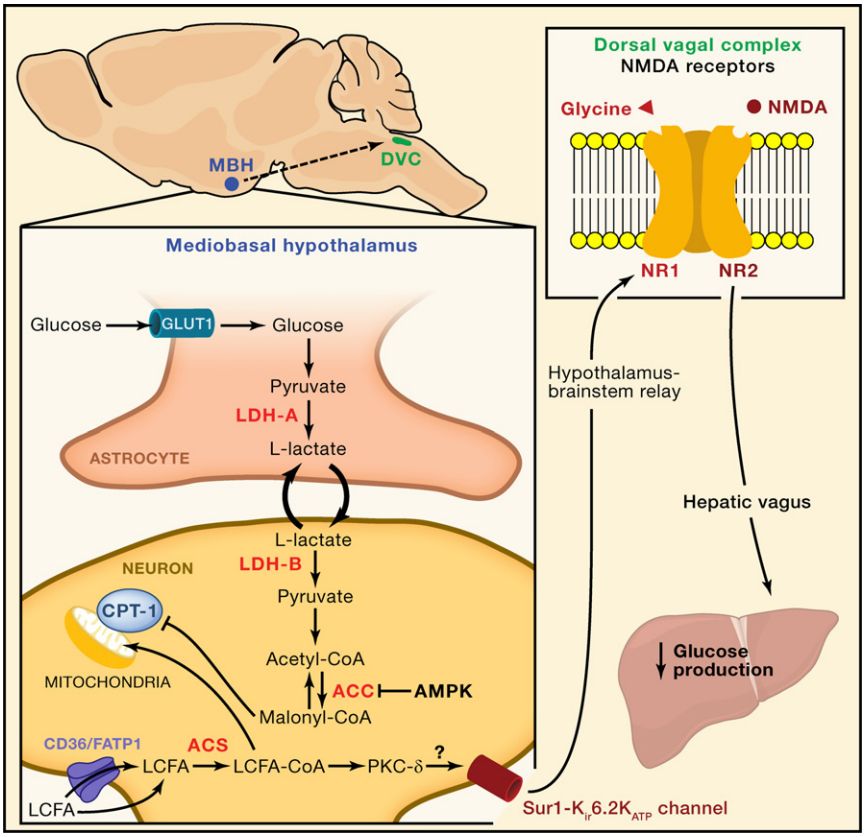

Figure 1. Proposed Model of Hypothalamic Lipid-Sensing Mechanisms

Long-chain fatty acids (LCFAs) are taken up by the brain via CD36 or FATP1 or passive diffusion. LCFAs are esterified by acyl-CoA synthetases (ACSs) to LCFA-CoAs, which enter mitochondria and undergo β oxidation via carnitine palmitoyltransferase-1 (CPT-1). Fatty acid oxidation is regulated by the availability of malonyl-CoA, which inhibits CPT-1 activity. The proposed model is that a short-term accumulation of cytosolic LCFA-CoAs activate PKC-δ and K_{ATP} channels which trigger neuronal transmission mediated by the NMDA receptors in the dorsal vagal complex (DVC) to inhibit hepatic glucose production in the presence of circulating basal insulin levels. In addition, hypothalamic LCFA-CoA sensing mechanisms mediate glucose-lactate metabolism to regulation glucose production. GLUT1, glucose transporter-1; LDH, lactate dehydrogenase.

cells and neurons, LCFAs are esterified to LCFA-CoA by acyl-CoA synthetase. Direct inhibition of hypothalamic acyl-CoA synthetase disrupts the ability of circulating fatty acids to accumulate hypothalamic LCFA-CoA (Lam et al., 2005b). As such, the inhibitory response on hepatic glycogenolysis to circulating fatty acid-stimulated hepatic gluconeogenesis (Chu et al., 2002; Chen et al., 1999; Lam et al., 2005b) is disrupted and leads to a dysregulation of glucose production (Lam et al., 2005b). Thus, circulating fatty acids are metabolized in the hypothalamus and that subsequent accumulation of fatty acid-derived LCFA-CoA is essential to trigger lipid-sensing mechanisms to inhibit glucose production and maintain glucose homeostasis in rodents.

During prolonged fasting, the level of plasma free fatty acids (Ruge et al., 2009) and the rate of hepatic glucogenogenesis (Landau et al., 1996) increase, while plasma glucose level remains at basal (Landau et al., 1996). Is the glucoregulatory impact of CNS lipid sensing relevant in prolonged fasting? First, rats undergone prolonged fasting have a significant reduction in hypothalamic LCFA-CoA level (He et al., 2006) in spite of an elevation of circulating fatty acid levels. Second, the relative contribution of hepatic gluconeogenesis to glucose production increases from 50%–90% as the duration of fasting increases (Landau et al., 1996). We postulate that hypothalamic lipid-sensing mechanisms are disrupted in prolonged fasting since hypothalamic LCFA-CoA level fails to accumulate. Consequently, the inhibitory effect on hepatic glycogenolysis diminishes over time, while fatty acid-stimulated hepatic gluconeogenesis becomes the primary contributor to glucose production and supplier of glucose fuel for the body. We propose that CNS lipid-sensing mechanisms are designed to trigger a negative feedback system to limit endogenous nutrient output in postprandial, but not in prolonged fasting, conditions.

A Malonyl-CoA → Carnitine Palmitoyltransferase-1 → LCFA-CoA Biochemical Sensory Axis

Carnitine palmitoyltransferase-1 (CPT-1) regulates the entry of LCFA-CoA into mitochondria for β oxidation (Caspi et al., 2007). Inhibition of hypothalamic CPT-1 elevates LCFA-CoA and inhibits glucose production in the presence of basal plasma insulin levels (Obici et al., 2003). The liver isoform of CPT-1, CPT-1a, is prevalent in the hypothalamus and the isoform that modifies glucose production (Obici et al., 2003). The brain-specific CPT-1 isoform CPT-1c (Price et al., 2002) is also expressed in the hypothalamic arcuate nucleus (Wolfgang et al., 2006) and implicated in the regulation of energy balance (Lane et al., 2008; Gao et al., 2011a; Wolfgang and Lane, 2011). The relative contribution of hypothalamic CPT-1c versus CPT-1a in glucose regulation remains to be assessed.

CPT-1 activity is endogenously inhibited by malonyl-CoA (McGarry et al., 1977; McGarry et al., 1983), and a rise in malonyl-CoA thus concurrently elevates LCFA-CoA (Caspi et al., 2007). Chronic reduction of hypothalamic malonyl-CoA levels via overexpression of malonyl-CoA decarboxylase induces hyperphagia and obesity (He et al., 2006; Hu et al., 2005) and in parallel negates the ability of circulating fatty acids to accumulate hypothalamic malonyl-CoA and LCFA-CoA levels to regulate glucose production (He et al., 2006). Although the relative role of hypothalamic malonyl-CoA and LCFA-CoA remains to be clarified, a hypothalamic malonyl-CoA → PT-1 → LCFA-CoA axis emerges to mediate CNS lipid sensing to regulate glucose and energy homeostasis (Figure 1).

Glucose enters the glial cells and elevates pyruvate and lactate (Bittar et al., 1996). Lactate is then shuttled into neurons and converted back to pyruvate to form acetyl-CoA and

generate fuel (Magistretti et al., 1993; Magistretti et al., 1999; Pellerin, 2010). In parallel, an enhancement of hypothalamic glucose flux into lactate → pyruvate → acetyl-CoA formation inhibits glucose production (Lam et al., 2005a), and plasma glucose levels in a nonclamp postprandial setting (Lam et al., 2005a). Given that acetyl-CoA is a precursor of malonyl-CoA that is enzymatically derived by acetyl-CoA carboxylase (ACC) (Lam et al., 2005c), the hypothalamic malonyl-CoA → CPT-1 → LCFA-CoA axis is positioned to mediate CNS glucose sensing to regulate peripheral glucose homeostasis. When hypothalamic ACC is inhibited through the activation of AMP-dependent protein kinase (AMPK), hypothalamic glucose and lactate sensing fail to inhibit glucose production (Yang et al., 2010). Conversely, inhibition of hypothalamic AMPK is sufficient to inhibit glucose production (Yang et al., 2010). Similarly, an enhancement of hypothalamic glucose flux into lactate and malonyl-CoA formation inhibits food intake (Cha and Lane, 2009; Lam et al., 2009), while central administration of citrate (activator of ACC) enhances the ability of circulating insulin to increase hepatic glycogen synthesis (Cesquini et al., 2008). These evidences support a potential critical role of the hypothalamic malonly-CoA → CPT-1 → LCFA-CoA sensory axis in mediating hypothalamic lipid and glucose sensing mechanisms to inhibit glucose production (Figure 1).

Protein Kinase C → ATP-Sensitive Potassium Channel

If the accumulation of hypothalamic LCFA-CoA is essential to mediate CNS lipid and glucose-sensing mechanisms, dissecting the downstream effectors of LCFA-CoA is central to unveil novel signaling molecules that inhibit glucose production and plasma glucose levels. LCFA-CoA and lipid-derived metabolite diacylglycerol activate isoforms of protein kinase C (PKC-δ, PCK-θ, and PKC-ε) and hinder the ability of circulating insulin to inhibit hepatic glucose production and stimulate muscle glucose uptake (Boden et al., 2005; Lam et al., 2002; Samuel et al., 2004; Shulman, 2000). The impact of hepatic PKC-δ on hepatic insulin action is recently confirmed in humans (Bezy et al., 2011). In the presence of basal plasma insulin levels, hypothalamic administration of PKC-activator 1-oleoyl-2-acetyl-sn-glycerol activates hypothalamic PKC-δ and inhibits glucose production (Ross et al., 2008). Conversely, inhibition of hypothalamic PKC-δ negates CNS lipid sensing to inhibit glucose production (Ross et al., 2008), supporting that activation of hypothalamic PKC-δ is sufficient and necessary for lipid sensing to inhibit glucose production. Of note, hypothalamic PKC-δ mediates the anorectic response of amphetamine (Kuo et al., 2009) and the ability of hypothalamic GLP-1 to alter peripheral glucose homeostasis (Cabou et al., 2011) as well.

PKC phosphorylates and activates the pore-forming subunit Kir6.2 of the ATP-sensitive potassium (K_{ATP}) channels in the pancreatic β cells (Light et al., 2000) of which the same K_{ATP} channels are expressed in hypothalamic neurons (Pocai et al., 2005a). Activation of the hypothalamic K_{ATP} channels is sufficient (Pocai et al., 2005a) and necessary for CNS lipid sensing (Lam et al., 2005b) to inhibit glucose production. Importantly, molecular and pharmacological inhibition of hypothalamic K_{ATP} channels negates the ability of hypothalamic PKC activator to lower glucose production as well (Ross et al., 2008). Thus, a hypothalamic LCFA-CoA → PKC-δ → K_{ATP} channels neuronal signaling axis emerges that potentially mediate lipid sensing (and highly likely glucose as well, although it remains to be tested) to inhibit glucose production (Figure 1). This is in parallel to the fact that hypothalamic PKC-δ and K_{ATP} channel activations are necessary for GLP-1 to regulate peripheral glucose homeostasis (Sandoval et al., 2008; Cabou et al., 2011). Although it is crucial to continue dissect the molecular, biochemical and neuronal mechanisms that underlie hypothalamic lipid sensing, it is equally important to assess whether extraneuronal and hypothalamic regions are necessary for hypothalamic lipid sensing to regulate glucose homeostasis. These studies will shed lights on potential novel signaling molecules that lower glucose levels.

A Hypothalamic-Hindbrain Neuronal Axis

The hindbrain receives neuronal projections from the hypothalamus to regulate feeding (Morton et al., 2006). Neurotransmission by *N*-methyl-D-aspartate (NMDA) receptors in the dorsal vagal complex (DVC) of the hindbrain relay ascending signals ignited by lipid sensing in the gut to maintain energy balance (Badman and Flier, 2005; Coll et al., 2007; Cummings and Overduin, 2007; Lam, 2010). Independent of changes in food intake, activation of NMDA receptors in the DVC is necessary for duodenal lipid sensing (Cheung et al., 2009; Kokorovic et al., 2011; Lam, 2010; Wang et al., 2008) and sufficient (Lam et al., 2010) to inhibit glucose production. In a nonclamp physiological setting, direct inhibition of DVC NMDA receptors negates the ability of nutrient sensing mechanisms activated by refeeding to maintain glucose homeostasis (Lam et al., 2011). These findings raise the possibility that NMDA receptor activation in the DVC is necessary for hypothalamic nutrient sensing mechanisms to inhibit glucose production. When tested, direct inhibition of DVC NMDA receptor-mediated transmission via molecular and pharmacological approaches negates the ability of hypothalamic lipid sensing mechanisms to inhibit glucose production (Lam et al., 2011), while hypothalamic lipid sensing stimulate neurons in the DVC (Pocai et al., 2005b) (Figure 1). It remains to be assessed whether the neuronal projection from the hypothalamus to DVC is direct or indirect through other nuclei such as the paraventricular hypothalamus since hypothalamic leucine metabolism triggers paraventricular hypothalamic oxytocine neurons to stimulate neurons in the DVC to inhibit food intake (Blouet et al., 2009).

The pancreatic clamp procedure that is used to evaluate the glucoregulatory impact of the hypothalamic-hindbrain lipid-sensing mechanisms involves the use of somatostatin to inhibit the endogenous insulin and glucagon secretion while plasma insulin levels are replaced at basal level. In these experimental conditions, it is to be acknowledged that plasma glucagon level is reduced by 30%–50%. However, direct activation of NMDA receptors in the DVC is still equally effective to inhibit glucose production when circulating glucagon is replaced at basal level via exogenous intravenous glucagon infusion (T.K.T.L., unpublished data).

In summary, a unifying lipid sensing pathway in the hypothalamus emerges that trigger a forebrain-hindbrain NMDA receptor-dependent signaling cascade to inhibit glucose production (Figure 1A). Although the physiological relevance of CNS lipid sensing in glucose regulation remains to be clarified, it is to be noted that in a nonclamp physiological setting, direct inhibition of NMDA receptor in the DVC disrupts glucose homeostasis during refeeding, and these findings support the working

hypothesis that DVC NMDA receptor is a master sensor to integrate nutrient-sensing mechanisms to maintain glucose homeostasis. In addition to being sensitive to nutrients, the brain is insulin responsive. We next direct our focus to studies that selectively evaluate the metabolic impact of insulin signaling in the brain.

Insulin Signaling in the Brain

Insulin Receptor and Action

A rise in circulating insulin levels inhibits hepatic glucose production and plasma glucose levels partly through direct binding to its receptor in the liver (Cherrington, 1999) and an inhibition of plasma free fatty acid levels (Bergman, 2007). Insulin receptors are expressed in the brain as well and central administration of insulin inhibits food intake (Woods et al., 1979). Conversely, neuron-specific insulin receptor knockout mice develop hyperphagia and insulin intolerant (Brüning et al., 2000).

Whole-body insulin resistance as seen in the neuron-specific insulin receptor knockout mice is attributed to the inability of a rise in circulating insulin level to inhibit glucose production as assessed by the hyperinsulinemic-euglycemic clamp of which somatostatin is not given (Fisher et al., 2005; Inoue et al., 2006). These glucoregulatory data are consistent with the fact that a relatively short-term knockdown of the hypothalamic insulin receptor negates the inhibitory effect on glucose production during a hyperinsuliemic-euglycemic clamp with somatostatin infusion in rats (Obici et al., 2002a). Selective inhibition of hypothalamic insulin receptor negates the ability of intracerebroventricular (icv) insulin administration to inhibit glucose production during the pancreatic (basal insulin) euglycemic clamps (Obici et al., 2002c), while icv insulin is equally potent to inhibit glucose production in mice in the same experimental conditions (Inoue et al., 2006).

In a nonclamp basal setting, direct administration of insulin into hypothalamus of rats leads to a marked and sustained lowering of plasma glucose level independent of a rise in systemic insulin level (Pocai et al., 2005a). Importantly, this glucose-lowering effect is seen as well in dogs as intracisternally insulin administration lowers blood glucose level (Chowers et al., 1966). The effect in dogs is later on reproduced and expanded by an independent group of which icv administration of insulin causes a marked and sustained hypoglycaemia in mongrel dogs (Agarwala et al., 1977). Strikingly, the central insulin-induced hypoglycaemia diminishes only when the liver is removed (Agarwala et al., 1977). Together, these data put forward a working hypothesis that insulin action in the brain lowers plasma glucose level through an inhibition of glucose production in rats and mice, and by modulation of hepatic glucose fluxes in dogs.

The ability of brain insulin action to regulate hepatic glucose fluxes in mongrel dogs is evaluated in two studies (Edgerton et al., 2006; Ramnanan et al., 2011). The first reports that a selective rise of insulin level in the head for 3 hr (versus control) fails to lower glucose production in the presence of systemic hyperinsulinemia and hepatic insulin deficiency, suggesting that the direct hepatic effect of insulin dominates the acute inhibitory effect on glucose production (Edgerton et al., 2006). The second study contains two sets of experiments of which the first illustrates that icv insulin infusion (like rodents; see below) activates hypothalamic AKT and hepatic STAT3 signaling, as well as inhibiting the expression of the gluconeogeneic enzymes glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (Ramnanan et al., 2011). However, icv insulin infusion only modestly inhibits net hepatic glucose output in the same experimental setting (Ramnanan et al., 2011). In the second set of experiments, insulin is directly infused toward the head for 6 hr via the arteries and elevates insulin level in the head, as well as in the artery and jugular vein in 42 hr fasted dogs. This protocol leads to a drop in circulating free fatty acid levels, which are prevented by concurrent Intralipid infusion. In these experimental conditions, insulin infusion toward the head inhibits the net hepatic glucose output by 4 hr of head infusion through an elevation of hepatic glucose uptake and glycogen synthesis, but with no changes in gluconeogenesis and glucose production (Ramnanan et al., 2011).

It is not the goal of this Perspective (although it is important) to discuss in details the possible sceneries and possibilities that underlie the discrepancies in the ability of insulin action in the brain to modulate glucose production in the rodents on one hand but to regulate hepatic glucose uptake (and inhibit net hepatic glucose output) in dogs on the other hand. Rather, it is to acknowledge that in light of the findings discussed above as a whole, central insulin action lowers plasma glucose levels and modulates hepatic glucose fluxes in a time-dependent fashions in rats, mice, and dogs. Based on this common ground, it is crucial to continue dissect the neuronal signaling mechanisms that mediate insulin's ability to regulate hepatic glucose fluxes in rodents and dogs. As such, novel signaling molecules that restore glucose production regulation and homeostasis in diabetes and obesity will be revealed since hypothalamic insulin resistance is a well-documented feature in high-fat diet-induced obese rodents (see below). It remains to be assessed whether hypothalamic insulin resistance occurs in high fat-fed dogs.

Insulin Signaling Pathway → K_{ATP} Channels → Hepatic Vagus → STAT3 Signaling

Hypothalamic insulin activates phosphatidylinositol-3 kinase (PI3K)-dependent signaling cascades in rats (Obici et al., 2002c) and dogs (Ramnanan et al., 2011) to modulate hepatic glucose fluxes. Downstream signaling effectors of hypothalamic insulin receptor substrate-2 and AKT mediate the ability of insulin to lower plasma glucose levels in diabetic rats (Gelling et al., 2006).

Insulin activates K_{ATP} channels in hypothalamic neurons of rats in vitro (Spanswick et al., 2000) and in the mediobasal hypothalamus of rats in vivo to inhibit glucose production (Obici et al., 2002c; Pocai et al., 2005a). Although the exact mechanism by which the insulin-PI3K signaling cascade induces K_{ATP} channel activation remains elusive, the hepatic vagal efferent innervation relays the neuronal signal from the brain to the liver to inhibit glucose production (Pocai et al., 2005a). This brain-liver axis also mediates the ability of hypothalamic lipid sensing to inhibit glucose production in the presence of basal insulin level (Pocai et al., 2005b) and the ability of hypothalamic leptin action to lower glucose production during a hyperinsulinemic-euglycemic clamp without somatostatin infusion in rodents (German et al., 2009). Importantly, a recent study has implicated that activation of hypothalamic K_{ATP} channels in normal humans lowers glucose production during the pancreatic basal insulin/glucagon clamps

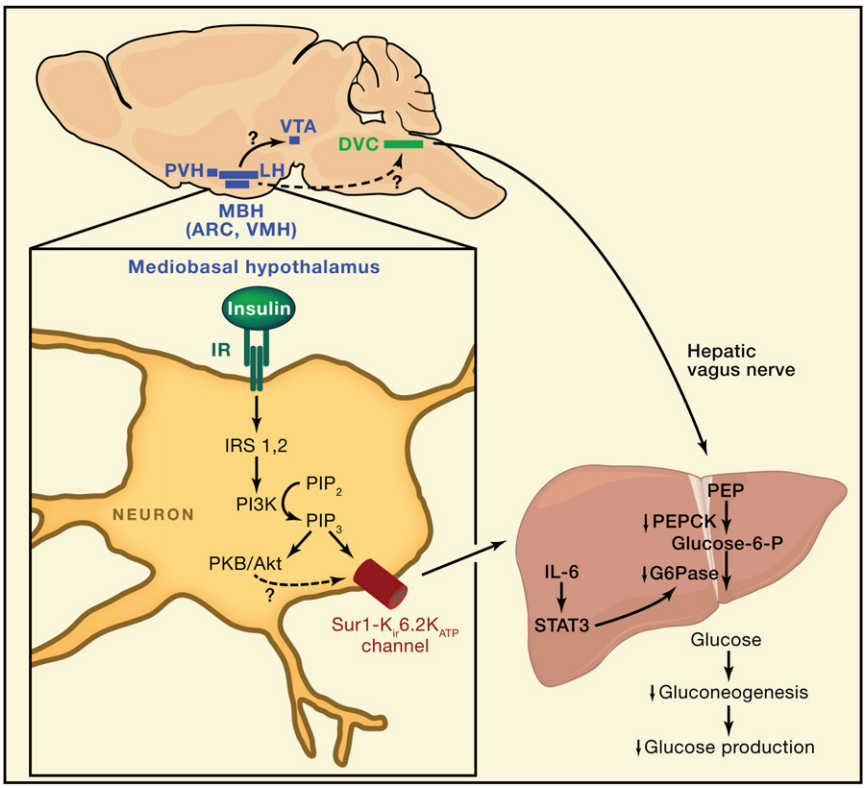

Figure 2. Proposed Model of Hypothalamic Insulin Signaling Pathways

Brain regions responsive to insulin include the mediobasal hypothalamus (MBH) (arcuate nucleus [ARC], ventromedial hypothalamus [VMH]), lateral hypothalamus (LH), paraventricular hypothalamus, and the ventral tegmental area (VTA) of the midbrain. Neuronal subpopulations responsive to insulin include ARC proopiomelanocortin (POMC) and neuropeptide Y (NPY)/Agouti-related peptide AgRP neurons, VMH steroidogenic factor 1 (SF-1) neurons, LH orexin neurons, PVH corticotrophin-releasing hormone (CRH) neurons, and VTA dopaminergic and catecholaminergic neurons. The proposed model is that insulin triggers signaling events in brain regions, particularly the MBH AgRP neurons, involving IRS1/2 → PI3K → PIP_3 to activate K_{ATP} channels. As such, neuronal signals are transmitted via the hepatic vagus to the liver to inhibit gluconeogenesis and glucose production through a hepatic IL-6-STAT3 dependent pathway. PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase.

(Kishore et al., 2011). Future experiments are warranted to address the discrepancies in the ability of the CNS to lower glucose production in normal rodents and humans versus the ability of the CNS to lower net hepatic glucose output via a stimulation of glucose uptake and glycogen synthesis in normal dogs.

Neurons expressing NPY and AgRP in the accurate nucleus are targets of hypothalamic insulin action (Schwartz et al., 1992; Könner et al., 2007). Insulin activates K_{ATP} channels and induces membrane hyperpolarization to reduce the firing rate of AgRP neurons (Könner et al., 2007). Knockout of insulin receptor selectively in the AgRP neurons negates the ability of circulating insulin to inhibit glucose production and hepatic gluconeogenic gene expressions, as well as stimulating hepatic IL-6 and STAT3 signaling, during a hyperinsulinemic-euglycemic clamp with no somatostatin given in rodents (Könner et al., 2007). Hepatic IL-6 and STAT3 signaling is central to the metabolic control of hypothalamic insulin action since icv insulin fails to lower glucose production in hepatic IL-6 and STAT3 knockout mice (Inoue et al., 2006). The activation of hepatic STAT3 is similarly postulated to mediate the ability of brain insulin action to stimulate hepatic glycogen synthesis in dogs (Ramnanan et al., 2011). These findings collectively indicate that insulin signaling in the AgRP neurons of the arcuate nucleus activates K_{ATP} channels and triggers the hepatic vagus to stimulate hepatic IL-6 and STAT3 signaling to inhibit glucose production in rodents (Figure 2). However, it is to be noted that the involvement of hepatic IL-6 signaling in mediating the ability of circulating hyperinsulinemia to inhibit glucose production remains to be clarified since recent studies indicate that hepatocyte-specific disruption of IL-6 signaling fails to alter the ability of hyerinsulinemic-euglycemic clamps to lower glucose production (Wunderlich et al., 2010)

Ventromedial Hypothalamus and Ventral Tegmental Area

The ventromedial hypothalamus (VMH) is insulin responsive (Spanswick et al., 2000; Davidowa and Plagemann, 2001; Cotero and Routh, 2009). Insulin activates PIP3 formation in genetically labeled SF-1 neurons in mice (Klöckener et al., 2011), and the VMH SF-1 neurons adjacent to the ventricle and accurate nucleus (i.e., mediobasal VMH) exhibits the strongest PIP3 activation (Klöckener et al., 2011). Insulin-PIP3 signaling activates K_{ATP} channels and inhibits firing of VMH SF-1 neurons (Klöckener et al., 2011). SF-1 neuron-specific deletion of insulin receptors in high fat-fed mice negates the chronic activation of insulin signaling in VMH SF-1 neurons, reverses obesity, and improves glucose and insulin tolerance (Klöckener et al., 2011). In contrast, genetic deletion of PI3K signaling in VMH SF-1 neurons predisposes mice to high-fat diet-induced obesity (Xu et al., 2010). Although studies are needed to clarify the ability of insulin signaling in VMH SF-1 neurons to control obesity, it would be important to evaluate whether VMH SF-1 neurons mediates insulin action to regulate hepatic glucose fluxes independent of changes in body weight.

Dopaminergic neurons in the midbrain ventral tegmental area (VTA) express insulin receptors (Figlewicz et al., 2003) and IRS-2 protein (Pardini et al., 2006). VTA insulin regulates dopamine reuptake via transcriptional control of dopamine transporters (Figlewicz et al., 1994) and influences food-finding behavior, food intake, and ultimately body weight (Figlewicz and Benoit, 2009). Mice with selective ablation of the insulin receptor gene in catecholaminergic neurons of the midbrain increase food intake and adiposity (Könner et al., 2011). The effect of insulin action in the VTA on the regulation of hepatic glucose fluxes, however, remains to be investigated.

In summary, these studies collectively put forward the hypothesis that insulin signaling in hypothalamic neurons lowers

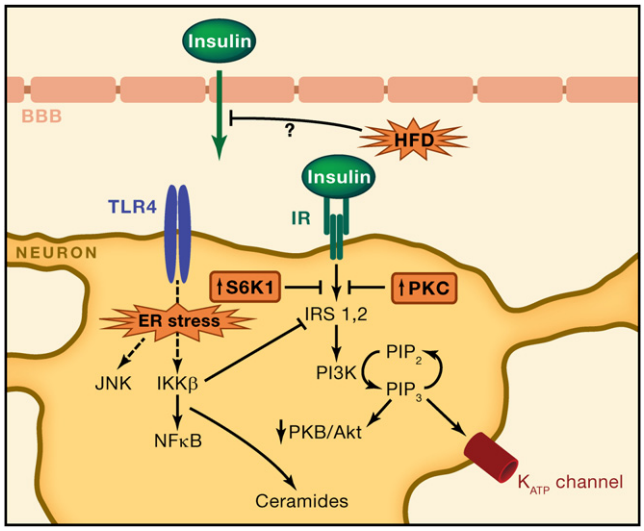

Figure 3. Proposed Model of High-Fat Diet-Induced Hypothalamic Insulin Resistance

High-fat feeding disrupts insulin-signaling pathways in the brain that regulate glucose and energy homeostasis. High-fat feeding is proposed to activate hypothalamic PKC-θ and/or p70 S6 kinase (S6K) and impair insulin to activate AKT to regulate energy and glucose homeostasis. In parallel, high-fat feeding activates hypothalamic ER stress and IKKβ/NF-kB and/or JNK signaling inflammatory pathways to impair insulin action while the role of hypothalamic Toll-like receptors (TLR) activation and ceramide synthesis could also play an important causative role in fat-induced hypothalamic insulin resistance. Orange-colored boxed elements represent high-fat feeding-induced changes.

plasma glucose levels by a modulation of hepatic glucose fluxes in normal condition. The important follow-up question then becomes whether high-fat feeding induces hypothalamic insulin resistance.

High-Fat Feeding and CNS Insulin Resistance

High-fat feeding renders a state of nutrient excess leading to pathophysiological conditions, such as obesity, insulin resistance, and type 2 diabetes. High-fat feeding leads to hepatic and muscle insulin resistance (Storlien et al., 1986; Wang et al., 2001; Shulman, 2000) and, more relevant to the scope of this Perspective, leads also to CNS insulin resistance.

Obesity impairs the transport of insulin through the blood-brain barrier (Israel et al., 1993; Kaiyala et al., 2000), but direct administration of insulin into the hypothalamus still fails to lower food intake (Clegg et al., 2011) and glucose production (Ono et al., 2008) in high fat-fed rodents. This is indicative of a direct impairment of hypothalamic insulin signaling in obesity (Belgardt and Brüning, 2010a).

The inability of central insulin to lower food intake in response to high-fat feeding associates with an impairment on hypothalamic AKT activation (Clegg et al., 2011) that is due partly to an activation of hypothalamic PKC-θ (Benoit et al., 2009). In parallel, high-fat feeding impairs the ability of hypothalamic insulin to inhibit glucose production through the activation of hypothalamic p70 S6 kinase (S6K) (Ono et al., 2008). S6K is phosphorylated by mTOR complex1 and inhibits AKT and insulin signaling (Dann et al., 2007). Overexpression of hypothalamic S6K is sufficient to negate the ability of central insulin to activate hypothalamic AKT and inhibit glucose production, while inhibition of hypothalamic S6K activity rescues the metabolic control of hypothalamic insulin action (Ono et al., 2008). Based on these observations, a working model becomes apparent that high-fat feeding disrupts hypothalamic insulin-AKT signaling in rodents, which in part could lead to hyperphagia and hyperglycemia.

An enhancement of endoplasmic reticulum (ER) stress and inflammation leads to peripheral insulin resistance in obesity (Hotamisligil, 2010). Similarly, high-fat feeding activates inflammatory processes in the rodent hypothalamus (De Souza et al., 2005) and hypothalamic IKKβ/NF-kB signaling inflammatory pathway (via elevated ER stress) to negate hypothalamic insulin action to lower food intake (Zhang et al., 2008). Importantly, activation of hypothalamic ER stress is sufficient to impair glucose tolerance and induce hepatic insulin resistance and hypertension (Purkayastha et al., 2011), while inhibition of CNS JNK signaling enhances insulin sensitivity and improves glucose tolerance upon high-fat feeding (Belgardt et al., 2010b) (Figure 3).

High-fat feeding induces hypothalamic inflammation and insulin resistance through the activation of the Toll-like receptors (TLRs) (Kleinridders et al., 2009), and again this phenomenon is in analogous to peripheral insulin resistance (Shi et al., 2006). In the peripheral tissues, fatty acid-induced ceramide biosynthesis is dependent on upstream TLR4 signaling and IKKβ activity (Holland et al., 2011), and that increased ceramide biosynthesis is required for TLR4-dependent insulin resistance (Holland et al., 2011). Ceramides are derived from saturated fatty acids and induce insulin resistance (Holland et al., 2007). Elevated biosynthesis of ceramides in the periphery contributes in the pathogenesis of obesity and the metabolic syndrome, and decreased circulating levels of ceramides achieved by inhibiting serine palmitoyltransferase, the enzyme that catalyzes the rate-limiting step of ceramides biosynthesis, improves the metabolic profiles of high-fat diet-induced obese mice (Yang et al., 2009). Of note, reduction of peripheral ceramide levels improves glucose homeostasis by enhancing hepatic insulin sensitivity (Yang et al., 2009).

In the hypothalamus, leptin-induced increases in malonyl-CoA and CPT-1c inhibition lead to an inhibition of the de novo synthesis of ceramide. The effect of ceramide synthesis appears to mediate the anoretic effect of leptin (Gao et al., 2011b). Given that leptin signaling (like insulin) in the hypothalamus enhances insulin action to inhibit glucose production (German et al., 2009), future studies are warranted to evaluate for a potential role of hypothalamic ceramide synthesis in the pathogenesis of hypothalamic insulin and leptin resistance.

In summary, based on studies to date, high-fat feeding induces hypothalamic insulin resistance through mechanisms that are shared in peripheral insulin resistance in rodents. Since hypothalamic insulin is postulated to trigger a similar brain-liver signaling axis in normal dogs to modulate hepatic glucose fluxes, it is essential to evaluate whether high-fat feeding induces hypothalamic insulin resistance in dogs.

High-Fat Feeding and CNS Lipid Sensing

In the presence of basal circulating insulin level, short-term accumulation of hypothalamic lipids signals through a LCFA-CoA → PKC-δ → K_{ATP}-dependent mechanism to inhibit glucose production in normal rodents as discussed above. Does high-fat feeding disrupt hypothalamic lipid sensing mechanisms?

High-fat feeding for 3 days disrupts the central short-term infusion of oleic acid to accumulate hypothalamic LCFA-CoA and inhibits food intake and glucose production in association with a stimulation of hypothalamic CPT-1 activity in the presence of basal circulating insulin levels (Pocai et al., 2006; Morgan et al., 2004). Direct inhibition of hypothalamic CPT-1 rescues the ability of CNS lipid-sensing mechanisms to elevate LCFA-CoA and inhibit glucose production and food intake in high fat-fed rats (Pocai et al., 2006), suggesting that the downstream signaling pathway of LCFA-CoA is intact in response to short-term high-fat feeding. In fact, direct activation of hypothalamic PKC-δ is equally potent to inhibit glucose production in the same short-term high fat-fed rats (Ross et al., 2008).

Of note, the hypothalamic malonyl-CoA → CPT-1 → LCFA-CoA axis mediates glucose and lactate sensing to inhibit glucose production in normal rodents. So, can direct enhancement of central lactate metabolism overcome LCFA sensing defects in high-fat feeding? Although the answer remains unknown, central administration of lactate is equally potent to lower glucose production in high fat-fed rats, as well as in uncontrolled diabetic rats in a nonclamp setting (Chari et al., 2008). Future studies are necessary to finely dissect the underlying nutrient-sensing mechanisms that regulate peripheral metabolic homeostasis in normal, diabetic, and obese conditions.

High-fat feeding for approximately 7 weeks has been documented to elevate hypothalamic palmityl-CoA and stearoyl-CoA (but not oleoyl-CoA) and impair the ability of central insulin injection to lower food intake (Posey et al., 2009). In addition, direct central administration of LCFAs on one hand lowers food intake and glucose production (Obici et al., 2002b) and on the other impairs the ability of central insulin and leptin action to inhibit food intake (Kleinridders et al., 2009; Milanski et al., 2009). The impact of short-term versus long-term effects of high-fat feeding on the accumulation of hypothalamic LCFA-CoAs and the ability of hypothalamic LCFA-CoAs to regulate peripheral glucose homeostasis under basal and insulin-stimulated conditions remain to be clarified.

Conclusion

In type 2 diabetes and obesity, a dysregulation of hepatic glucose production and feeding leads to a disruption in metabolic homeostasis. Independent lipid-sensing (Figure 1) and insulin-signaling (Figure 2) pathways in the hypothalamus are proposed to relay signals to various brain nuclei and trigger a brain-liver axis to inhibit glucose production in normal conditions.

High-fat feeding concurrently disrupts hypothalamic insulin-signaling (Figure 3) and lipid-sensing mechanisms at various sites that led to a breakdown of metabolic homeostasis. Importantly, studies that aimed to restore hypothalamic insulin signaling and lipid sensing are able to partly restore glucose and energy homeostasis in diabetes and obesity. As new studies emerge that evaluate the impact on peripheral nutrient metabolism when cellular and neuronal nutrient sensing and hormonal signaling pathways are manipulated, new knowledge will be gained with the hope that information can be integrated across mammals such that better medicine can be derived for diabetes and obesity.

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